(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 March 2003 (06.03.2003)

PCT

(10) International Publication Number
WO 03/018026 A1

(51) International Patent Classification[7]: A61K 31/565, 31/57, A61P 5/30

(21) International Application Number: PCT/NL02/00333

(22) International Filing Date: 23 May 2002 (23.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01203305.6 31 August 2001 (31.08.2001) EP
01204377.4 15 November 2001 (15.11.2001) EP

(71) Applicant *(for all designated States except US)*: **PANTARHEI BIOSCIENCE B.V.** [NL/NL]; P.O. Box 464, NL-3700 AL Zeist (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COELINGH BENNINK, Herman, Jan, Tijmen** [NL/NL]; Melvill van Carnbeelaan 38, NL-3971 BE Driebergen (NL). **BUNSCHOTEN, Evert, Johannes** [NL/NL]; Plantsoen 16, NL-5384 EV Heesch (NL). **HOLINKA, Christian, Franz** [US/US]; 299 West 12th Street - 97, New York, NY 10014 (US).

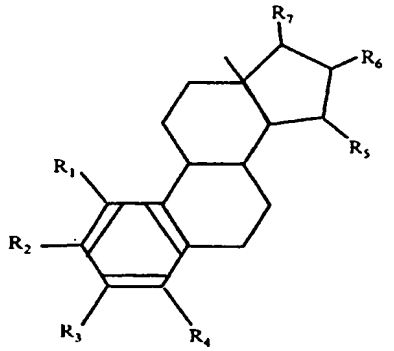
(74) Agents: **JORRITSMA, Ruurd** et al.; Nederlandsch Octrooibureau, Scheveningsweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: USE OF ESTROGENIC COMPOUNDS IN COMBINATION WITH PROGESTOGENIC COMPOUNDS IN HORMONE-REPLACEMENT THERAPY



(I)

(57) Abstract: One aspect of the present invention relates to method of hormone replacement in mammals, which method comprises the parenteral or rectal administration of an estrogenic component and a progestogenic component to a mammal in an amount effective to treat or prevent symptoms of hypoestrogenism, wherein the estrogenic component is selected from the group consisting of: substances represented by the following formula (I) in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alokxy group with 1-5 carbon atoms; each of R_5, R_6, R_7, is a hydroxyl group; and no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms; precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and mixtures of one or more of the aforementioned substances and/or precursors. Another aspect of the invention concerns a drug delivery system for parenteral or rectal administration that contains the aforementioned estrogenic component and an androgenic component, said drug delivery system being selected from the group consisting of suppositories, systems for intravaginal delivery, inhalers, nasal sprays and transdermal delivery systems.

WO 03/018026 A1

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

USE OF ESTROGENIC COMPOUNDS IN COMBINATION WITH PROGESTOGENIC COMPOUNDS IN HORMONE-REPLACEMENT THERAPY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of hormone replacement in mammals. More particularly the invention is concerned with a method of hormone replacement that comprises the parenteral or rectal administration of a combination of an estrogenic component and a progestogenic component in an effective amount to prevent or treat symptoms of hypoestrogenism.

BACKGROUND OF THE INVENTION

In hormone replacement therapy (HRT), sometimes also referred to as estrogen replacement therapy, estrogens are administered to prevent or treat symptoms resulting from estrogen deficiency or hypoestrogenism. Hypoestrogenism can occur in both females and males, and can lead to disorders and ailments such as osteoporosis (loss of bone mass), arteriosclerosis, climacteric symptoms such as hot flushes (flashes), sweats, urogenital atrophy, mood disturbances, insomnia, palpitations. Estrogen deficiency has also been associated with cognitive disturbances and Alzheimer's disease.

Hypoestrogenism, and in particular chronic hypoestrogenism, is frequently observed in (peri-)menopausal and post-menopausal women. However, it can also result from hypogonadism or castration, as well as from primary ovarian failure, treatment of e.g. breast cancer with aromatase inhibitor and gonadotropin-releasing hormone analogue treatment of benign gynaecological diseases such as endometriosis, adenomyosis, uterine fibroids (leiomyomas), dysmenorrhoea, menorrhagia and metrorrhagia.

HRT employs continuous administration of effective amounts of an estrogen for prolonged periods of time. The administration of estrogens has been associated, however, with endometrial proliferation in women and it is now widely accepted that "unopposed" estrogen therapy substantially increases the risk of endometrial cancer (Cushing et al., 1998. Obstet. Gynecol.91, 35-39; Tavani et al., 1999. Drugs Aging, 14, 347-357). There is also evidence of a significant increase in breast cancer with long-term (10-15 years) use of

estrogen therapy (Tavani et al., 1999. Drugs Aging, 14, 347-357; Pike et al., 2000. Steroids, 65, 659-664).

In order to counteract the negative effects of unopposed estrogen therapy, adjunctive progestogen treatment is nowadays commonly applied. Regular progestogen administration is believed to inhibit the continual estrogen stimulation of the endometrium through an anti-proliferative effect and appears to reduce the incidence of endometrial carcinoma in post-menopausal women receiving estrogen replacement therapy (Beral et al., 1999. J. Epidemiol. Biostat., 4, 191-210). Such an adjunctive treatment, generally using synthetic progestogens, is given either in continuous combined regimens with estrogen, or added sequentially, typically for about 14 days each month, to continuous estrogen treatment.

Endogenous and exogenous estrogens fulfil important central nervous and metabolic functions in the female organism: normal estrogen levels make a decisive contribution to a woman's well-being. Notwithstanding the widespread use of estrogens in HRT methods, there are still some unsolved problems. Known estrogens, in particular the biogenic estrogens (i.e. estrogens naturally occurring in the human body), are eliminated from the blood stream very quickly. For instance, for the main human biogenic estrogen 17β-estradiol the half-life is around 1 hour. As a result, between separate administration events, blood serum levels of such biogenic estrogens tend to fluctuate considerably. Thus, shortly after administration the serum concentration is usually several times higher than the optimum concentration. In addition, if the next administration event is delayed, serum concentrations will quickly decrease to a level where the estrogen is no longer physiologically active.

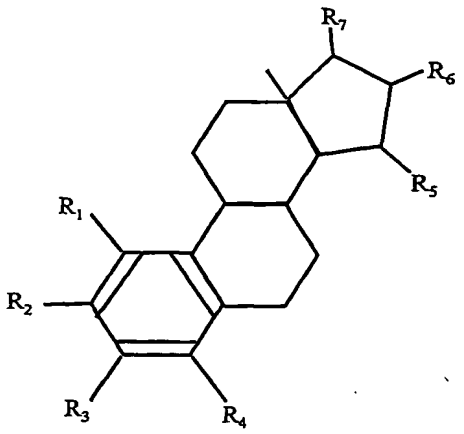
The most important synthetically altered estrogenic steroid is 17α-ethinyl estradiol (EE). This estrogen is dominant in oral hormonal contraception and is hardly used in HRT methods because prolonged administration of EE has been asociated with an increased risk of thromboembolism, which deemed to be particularly detrimental in menopausal and postmenopausal females. Apart from EE, mestranol has been used in a few cases; mestranol is a "prodrug" that is metabolised to EE in the organism. The liver is a target organ for estrogens. The secretion activity that is affected by estrogens in the human liver includes increased synthesis of transport proteins CBG, SHBG, TBG, several factors that are important for the physiology of blood clotting, and lipoproteins. The strong hepatic estrogenicity of ethinyl estradiol and diethylstilbestrol (DES), especially their effect on haemostasis factors, may explain why these synthetic estrogens have been associated with the enhanced risk of thromboembolism. Other undesirable side-effects that have been reported in relation to the

use of synthetic estrogens include fluid retention, nausea, bloating, cholelithiasis, headache and breast pain.

The aforementioned deficits are of considerable clinical significance when commonly known biogenic or synthetic estrogens are applied. Consequently, there is an as yet unmet need for estrogens that do not display these deficits and which can suitably be employed in HRT methods to effectively replace endogenous ovarian secretion of estradiol, i.e. to treat or prevent symptoms of hypoestrogenism.

SUMMARY OF THE INVENTION

The inventors have surprisingly found that these objectives are met by estrogenic substances that are represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms; each of R_5, R_6, R_7 is a hydroxyl group; and no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms.

A known representative of this group of estrogenic substances is 1,3,5 (10)-estratrien-3, 15α,16α,17β-tetrol, also known by the names of estetrol, oestetrol and 15α-hydroxyestriol. Estetrol is an estrogen that is produced by the fetal liver during human pregnancy. Unconjugated estetrol levels in maternal plasma peak at about 1.2 ng/ml at term pregnancy and are about 12 times higher in fetal than in maternal plasma (Tulchinsky et al., 1975. J. Clin. Endocrinol. Metab., 40, 560-567).

In 1970, Fishman et al., "Fate of 15α-hydroxyestriol-^{3}H in Adult Man", J Clin Endocrinol Metab (1970) 31, 436-438, reported the results of a study wherein tritium labeled 15α-hydroxyestriol (estetrol) was administered intravenously to two adult women. It was found that the estetrol was rapidly and completely excreted in urine as the glucosiduronate and that virtually no metabolism except for conjugation took place.

Between 1975 and 1985 several researchers have investigated the properties of estetrol and reported on its estrogenic potency and uterotrophic activity. The most relevant publications that were issued during this period are mentioned below:

- Levine et al., 1984. Uterine vascular effects of estetrol in nonpregnant ewes. Am. J. Obstet. Gynecol., 148:73, 735-738: "When intravenously administered in nonpregnant ewes, estetrol is 15 to 30 times less potent than estriol and 17β-estradiol in uterine vasodilation".
- Jozan et al., 1981. Different effects of oestradiol, oestriol, oestetrol and of oestrone on human breast cancer cells (MCF-7) in long term tissue culture. Acta Endocrinologica, 98, 73-80: "Estetrol agonistic potency is 2% of the magnitude observed for 17β-estradiol in in vitro cell proliferation".
- Holinka et al., 1980. Comparison of effects of estetrol and tamoxifen with those of estriol and estradiol on the immature rat uterus. Biol. Reprod. 22, 913-926: "Subcutaneously administered estetrol has very weak uterotrophic activity and is considerable less potent than 17β-estradiol and estriol".
- Holinka et al., 1979. In vivo effects of estetrol on the immature rat uterus. Biol. Reprod. 20, 242-246: "Subcutaneously administered estetrol has very weak uterotrophic activity and is considerable less potent than 17β-estradiol and estriol".
- Tseng et al., 1978. Heterogeneity of saturable estradiol binding sites in nuclei of human endometrium. Estetrol studies. J. Steroid Biochem. 9, 1145-1148: "Relative binding of estetrol to estrogen receptors in the human endometrium is 1.5 % of 17β-estradiol".
- Martucci et al., 1977. Direction of estradiol metabolism as a control of its hormonal action-uterotrophic activity of estradiol metabolites. Endocrin. 101, 1709-1715: "Continuous administration of estetrol from a subcutaneous depot shows very weak uterotrophic activity and is considerably less potent than 17β-estradiol and estriol".
- Tseng et al., 1976. Competition of estetrol and ethynylestradiol with estradiol for nuclear binding in human endometrium. J. Steroid Biochem. 7, 817-822: "The relative binding

constant of estetrol binding to the estrogen receptor in the human endometrium is 6.25% compared to 17β-estradiol (100%)".

- Martucci et al., 1976. Uterine estrogen receptor binding of catecholestrogens and of estetrol (1,3,5(10)-estratriene-3,15alpha,16alpha, 17beta-tetrol). Steroids, 27, 325-333: "Relative binding affinity of estetrol to rat uterine cytosol estrogen receptor is 0.5% of 17β-estradiol (100%). Furthermore, the relative binding affinity of estetrol to rat uterine nuclear estrogen receptor is 0.3% of 17β-estradiol (100%)".

All of the above publications have in common that the authors have investigated the estrogenic potency of estetrol. Without exception they all conclude that estetrol is a weak estrogen. In some of the cited articles the estrogenic potency of estetrol has been found to be lower than that of another biogenic estrogen, namely, 17β-estradiol, which is considered to be a relatively weak estrogen (e.g. compared to ethinyl estradiol). With these findings in mind, it is not surprising that the interest in estetrol has dwindled since the early eighties and that no publications on the properties of estetrol have been issued since.

US 5,468,736 (Hodgen) describes a method of hormone replacement therapy involving the administration of estrogen together with an amount of antiprogestin (antiprogestogen), which inhibits estrogen-induced endometrial proliferation in women. In Example 3 the combined use of estetrol and lilopristone is mentioned. No clues are given in the examples as to the mode and frequency of administration or regarding the dosage level employed. A disadvantage associated with the use of antiprogestogens, such as lilopristone, is the risk of inducing abnormal endometrial morphology, i.e. cystic hyperplasia, as has been observed in women who received an antiprogestogen treatment against endometriosis (Murphy et al., 1995. Fertil. Steril., 95, 761-766).

US 5,340,586 (Pike et al.) is concerned with compositions and methods which are effective to treat oophorectomised women, wherein an effective amount of an estrogenic composition and an androgenic composition are provided over a period of time. In the US-patent it is stated that natural and synthetic estrogenic compositions that can be used include natural estrogenic hormones and congeners, including but not limited to estradiol, estradiol benzoate, estradiol cypionate, estradiol valerate, estrone, diethylstilbestrol, piperazine estrone sulfate, ethinyl estradiol, mestranol, polyestradiol phosphate, estriol, estriol hemisuccinate, quinestrol, estropipate, pinestrol and estrone potassium sulfate, and furthermore that equine estrogens, such as equilelinin, equilelinin sulfate and estetrol, may also be employed. Except

for the exhaustive inventory of known estrogens, no other reference to estetrol (which is erroneously referred to as an equine estrogen) is made in this US-patent.

The same exhaustive list of estrogens is found in the following patent documents:

- US 4,762,717 (Crowley): A contraceptive method comprising the sequential administration of (1) a combination of luteinizing hormone releasing hormone (LHRH) and estrogen and (2) a combination of LHRH and estrogen and progestogen.
- US 5,130,137 (Crowley): A method of treating benign ovarian secretory disorder comprising the sequential administration of (1) a combination of luteinizing hormone releasing hormone (LHRH) and estrogen and (2) a combination of LHRH and estrogen and progestogen.
- US 5,211,952 (Spicer et al.): A contraceptive method comprising administering a gonadotropin hormone releasing hormone (GnRH) composition in an amount effective to inhibit ovulation and administering estrogen and progestogen to maintain serum levels above a defined minimum level.
- US 5,340,584 (Spicer et al.): A method for preventing conception or for treating benign gynaecological disorders comprising administering a GnRH composition for a first period of time in an amount effective to suppress ovarian estrogen and progesterone production, simultaneously administering an estrogenic composition in an amount effective to prevent symptoms of estrogen deficiency and simultaneously administering a progestogen in an amount effective to maintain serum level of said progestogen at a level effective to decrease endometrial cell proliferation.
- US 5,340,585 (Pike et al.): A method of treating benign gynaecological disorders in a patient in whom the risk of endometrial stimulation by estrogenic compositions is minimised or absent, comprising administering a GnRH composition in an amount effective to suppress ovarian estrogen and progesterone production and administering an estrogenic composition in an amount effective to prevent symptoms of estrogen deficiency.
- WO 00/73416 (Yifang et al.): A method for regulating the fertility of a host, comprising contacting host ovarian cells with a safe and effective amount of a pharmaceutical composition comprising an antisense oligonucleotide that is complementary to the nucleotide sequence of the follicle stimulating hormone (FSH) receptor. The possibility of combined administration of such an antisense oligonucleotide with an estrogenic steroid is mentioned in the application.

The benefits of the present invention may be realised without the co-administration of anti-progestogens, LHRH compositions, GnRH compositions and/or antisense oligonucleotides that are complementary to the nucleotide sequence of the follicle stimulating hormone (FSH) receptor as proposed in the aforementioned publications. Also, the present invention may suitably be applied in individuals who have not been oophorectomised, or in whom the risk of endometrial stimulation by estrogenic compositions is not minimised or absent, other than through the co-administration of a progestogen. Furthermore the present method does not require the use of a slow release formulation as is dictated by most of the aforementioned US-patents.

In view of the low estrogenic potency of the estetrol-like substances that are employed in accordance with the invention, it is surprising that these substances can effectively be used in a method of hormone replacement. Although the inventors do not wish to be bound by theory, it is believed that the unexpected efficacy of parenterally or rectally administered estetrol-like substances results from the combination of unforeseen favourable pharmacokinetic (ADME) and pharmacodynamic properties of these substances.

As regards the pharmacokinetic properties of the present estrogenic substances the inventors have discovered that their *in vivo* half-life is considerably longer than that of other biogenic estrogens. Thus, even though estetrol and estetrol-like substances have relatively low estrogenic potency, they may effectively be employed in HRT methods because their low potency is compensated for by a relatively high metabolic stability, as demonstrated by a long half-life.

An advantageous property of the present estrogenic substances resides in the fact that sex hormone-binding globulin (SHBG) hardly binds these estrogenic substances, meaning that, in contrast to most known estrogens, serum levels are representative for bio-activity and independent of SHBG levels.

Another important benefit of the present estrogenic substances is derived from their relative insensitivity to interactions with other drugs (drug-drug interactions). It is well known that certain drugs may decrease the effectiveness of estrogens, such as ethinyl estradiol, and other drugs may enhance their activity, resulting in possible increased side-effects. Similarly estrogens may interfere with the metabolism of other drugs. In general, the effect of other drugs on estrogens is due to interference with the absorption, metabolism or excretion of these estrogens, whereas the effect of estrogens on other drugs is due to competition for metabolic pathways.

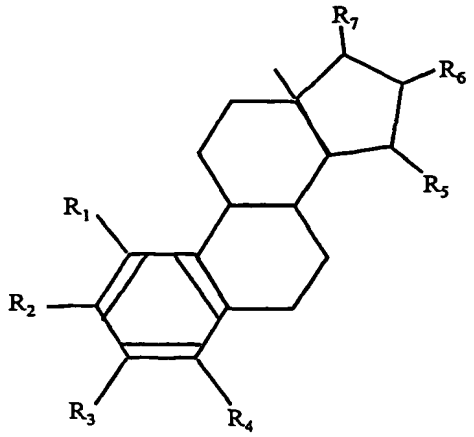
The clinically most significant group of estrogen-drug interactions occurs with drugs that may induce hepatic microsomal enzymes which may decrease estrogen plasma levels below therapeutic level (for example, anticonvulsant agents; phenytoin, primidone, barbiturates, carbamazepine, ethosuximide, and methosuximide; antituberculous drugs such as rifampin; antifungal drugs such as griseofulvin). The present estrogenic substances are less dependent on up- and downregulation of microsomal liver enzymes (e.g. P450's) and also are less sensitive to competition with other P450 substrates. Similarly, they do not interfere significantly in the metabolism of other drugs.

The conjugates of most estrogens, as formed in the liver, are excreted in the bile and may be broken down by gut bacteria in the colon to liberate the active hormone which can then be reabsorbed (enterohepatic recirculation). There are clinical reports that support the view that enterohepatic recirculation of estrogens decreases in women taking antibiotics such as ampicillin, tetracycline, etc. Conjugated forms of the present estrogenic substances are hardly excreted in the bile, meaning that they are substantially insensitive to drugs that do influence the enterohepatic recirculation of other estrogens.

The above observations serve to explain why the estrogenic substances of the invention hardly suffer from drug-drug interactions and thus produce a very consistent, i.e. predictable, impact. Thus, the efficacy of the estrogenic substances of the invention is highly reliable.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly one aspect of the present invention relates to a method of hormone replacement in mammals, which method comprises the parenteral or rectal administration of an estrogenic component and a progestogenic component to a mammal in an amount effective to treat or prevent symptoms of hypoestrogenism, wherein the estrogenic component is selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms; each of R_5, R_6, R_7 is a hydroxyl group; and no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms;

precursors capable of liberating a substance according to the aforementioned formula when used in the present method;

and mixtures of one or more of the aforementioned substances and/or precursors. The term "parenteral administration" as used in here encompasses transdermal, intranasal, intravaginal, pulmonary, buccal, subcutaneous, intramuscular and intra-uterine administration.

The HRT method according to the invention may advantageously be used to treat all known forms of hypoestrogenism, e.g. hypoestrogenism associated with (peri-)menopausal and post-menopausal women, hypoestrogenism resulting from hypogonadism or castration, as well as hypoestrogenism resulting from primary ovarian failure, treatment of e.g. breast cancer with aromatase inhibitor and gonadotropin-releasing hormone analogue treatment of e.g. benign gynaecological diseases. Examples of manifestations of hypoestrogenism that can effectively be treated or prevented with the present method in both females and males include osteoporosis, arteriosclerosis, cognitive disturbances and Alzheimer's disease. The method may also advantageously be used in the (prophylactic) treatment of climacteric symptoms such as hot flushes (flashes), sweats, urogenital atrophy, mood disturbances, insomnia and palpitations. The present method is particularly suited for treating or preventing osteoporosis and climacteric symptoms.

The term "estrogenic component" as used throughout this document encompasses substances that are capable of triggering an estrogenic response *in vivo*, as well as precursors that are capable of liberating such an estrogenic component *in vivo* when used in accordance

with the present invention. In order for estrogenic components to trigger such a response they normally have to bind to an estrogen receptor, which receptors are found in various tissues within the mammalian body. The term "progestogenic component" is defined as a substance that is capable of triggering an progestogenic response *in vivo* or a precursor which is capable of liberating such a substance *in vivo*. Usually progestogenic components are capable of binding to a progestogen receptor.

It is noted that the present invention not only encompasses the use of estrogenic and progestogenic components specifically mentioned in this application, but also metabolites of these hormones that display comparable *in vivo* functionality. In this context it is observed that, for instance, levonorgestrel is a metabolite of norgestimate and that estriol is a metabolite of 17beta-estradiol. Both these progestogens and estrogens have found application in contraceptive formulations and/or hormone replacement therapy. The term "estrogenic substances" as used in this document does not encompass tritium (^3H) labeled estrogenic substances such as tritium labeled estetrol.

The present estrogenic substances are distinct from both the biogenic and synthetic estrogens that are commonly applied in pharmaceutical formulations in that they contain at least 4 hydroxyl groups. The present substances are special in that the 5 membered ring in the steroid skeleton comprises 3 hydroxyl substituents rather than 0-2.

Known estrogens that contain at least 4-hydroxyl groups and derivatives thereof are:

1, 3, 5(10)-estratrien-2, 3, 15α, 16α, 17β- pentol 2-methyl ether

1, 3, 5(10)-estratrien-2, 3, 15β, 16α, 17β- pentol 2-methyl ether

1, 3, 5(10)-estratrien-2, 3, 16α, 17β- tetrol

1, 3, 5(10)-estratrien-3, 4, 16α, 17β- tetrol 4-methyl ether

1, 3, 5(10)-estratrien-3, 15α, 16α, 17β- tetrol

1, 3, 5(10)-estratrien-3, 15α, 16α, 17β- tetrol tetra acetate

1, 3, 5(10)-estratrien-3, 15β, 16β, 17β- tetrol tetra acetate

Preferably, the estrogenic substance applied as the active component in the present composition is a natural estrogen, i.e. an estrogen that is found in nature and especially in mammals. Even more preferably, the estrogenic substance is a so called biogenic estrogen, i.e. an estrogen that occurs naturally in the human body, a precursor of a biogenic estrogen or mixtures thereof. Because biogenic estrogens are naturally present in the fetal and female body, side-effects are not expected to occur, particularly not if the serum levels resulting from the exogenous administration of such estrogens do not substantially exceed naturally occurring concentrations. Since estetrol serum levels in the fetus are several times higher than

those found in pregnant females and knowing that the fetus is particularly vulnerable, estetrol is deemed to be a particularly safe biogenic estrogen. Side-effects are not expected to occur, particularly not if the serum levels resulting from the exogenous administration of such estrogens do not substantially exceed naturally occurring (fetal) concentrations. With synthetic estrogens such as ethinyl estradiol there is a (dose dependent) risk of undesirable side-effects, such as thromboembolism, fluid retention, nausea, bloating, cholelithiasis, headache and breast pain.

In a preferred embodiment of the present invention the estrogenic substance contains 4 hydroxyl groups. Also, in the aforementioned formula, R_1 preferably represents a hydrogen atom. In said formula preferably at least 2, more preferably at least 3 of the groups R_1, R_2, R_3 and R_4 represent a hydrogen atom.

The estrogenic substances according to the formula encompass various enantiomers since the carbon atoms that carry hydroxyl-substituents R_5, R_6 and R_7 are chirally active. In one preferred embodiment, the present estrogenic substance is 15α-hydroxy substituted. In another preferred embodiment the substance is 16α-hydroxy substituted. In yet another preferred embodiment, the substances is 17β-hydroxy substituted. Most preferably the estrogenic substances are 15α,16α,17β-trihydroxy substituted.

In another preferred embodiment of the present invention R_3 represents a hydroxyl group or an alkoxy group. In another preferred embodiment the groups R_1, R_2 and R_4 represent hydrogen atoms, in which case, if R_3, R_5, R_6 and R_7 are hydroxyl groups, the substance is 1,3,5 (10)-estratrien-3, 15,16,17-tetrol. A preferred isomer of the latter substance is 1,3,5 (10)-estratrien-3, 15α,16α,17β-tetrol (estetrol).

The invention also encompasses the use of precursors of the estrogenic substances that constitute the active component in the present method. These precursors are capable of liberating the aforementioned estrogenic substances when used in the present method, e.g. as a result of metabolic conversion. These precursors are preferably selected from the group of androgenic precursors as well as derivatives of the present estrogenic substances. Suitable examples of androgenic precursors include androgens that can be converted into the present estrogenic substances through *in vivo* aromatisation. Examples of derivatives of the present estrogenic substances that can suitably be used as precursors include such substances wherein the hydrogen atom of at least one of the hydroxyl groups has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic acid or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranal; or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue.

Typical examples of precursors which can suitably be used in accordance with the invention are esters that can be obtained by reacting the hydroxyl groups of the estrogenic substances with substances that contain one or more carboxy (M^+ $^-$OOC-) groups, wherein M^+ represents a hydrogen or (akali)metal cation. Hence, in a particularly preferred embodiment, the precursors are derivatives of the estrogenic substances, wherein the hydrogen atom of at least one of the hydroxyl groups in said formula has been substituted by -CO-R, wherein R is a hydrocarbon radical comprising from 1-25 carbon atoms. Preferably R is hydrogen, or an alkyl, alkenyl or aryl radical comprising from 1-20 carbon atoms.

The present method usually employs uninterrupted parenteral or rectal administration of the estrogenic component during a period of at least 10 days, preferably of at least 20 days.

The term "uninterrupted" as used in here, means that the estrogenic component is administered at relatively regular intervals, with no (therapeutically) significant interruptions. Naturally, minor interruptions may occur that do not affect the overall effectiveness of the present method, and indeed such aberrations are encompassed by the present invention. In a preferred embodiment, and more arithmetically, the administration regimen is deemed to be continuous if the longest interval between 2 subsequent administrations is not more than 3.5 times as long as the average interval. Even more preferably said longest interval is not more than 2.5 times, most preferably not more than 1.5 times as long as the average interval.

The benefits of the present invention are most pronounced when the estrogenic component is used in longer term hormone replacement therapy so as to minimise the negative effects of chronic hypoestrogenism. Therefore, the method of hormone replacement therapy, preferably, comprises administering the estrogenic component for a period of at least 1 month, more preferably of at least 3 months.

In the present method, the estrogenic and progestogenic component may be administered in separate dosage units. However, it is also possible and indeed very convenient to combine these two components into a single dosage unit.

The invention may suitably be reduced to practice in the form of a variety of HRT methods that are known to the person skilled in the art. Amongst these methods are the so called "combined" methods. The combined methods make use of preparations that contain a combination of an estrogen and a progestogen. The combined methods have in common that they are based on a regimen which involves administration of the aforementioned combined preparation, followed by an administration-free interval of about 7 days whereby withdrawal bleeding, simulating the natural menses, occurs. Thus 21 day intervals of hormone administration alternate with 7 days during which no hormones are administered.

As an alternative to the aforementioned combined methods, the so called "sequential" method has been proposed. Typical of the sequential method is that it comprises two consecutive phases, i.e. one phase during which estrogen and no progestogen is administered and another phase during which a combination of estrogen and progestogen is administered. The first sequential methods, like the aforementioned combined methods, made use of an administration free interval of about 7 days. More recently, sequential methods have been proposed which do not include an administration-free (or placebo) period, meaning that estrogen is administered throughout the full cycle and that progestogen is co-administered during only part of that cycle. WO 95/17895 (Ehrlich et al.) describes such an uninterrupted sequential method.

Yet another example of an HRT method which is encompassed by the present invention is the so called "continuous combined" method, which is a particular version of the combined method that uses uninterrupted combined administration of a progestogenic and an estrogenic component during a prolonged period of time, e.g. more than 50 days. In contrast to ordinary combined and sequential methods, no regular menses occur in the continuous combined method as the continuous administration of progestogen in the indicated amounts induces amenorrhoea.

In one embodiment of the invention, which relates to the continuous combined method, the present method comprises the uninterrupted parenteral or rectal administration of the combination of the estrogenic component and the progestogenic component during a period of at least 28, preferably at least 60 days.

In another embodiment of the invention, which relates to sequential and combined methods that employ a significant administration-free interval, the method of the invention comprises an interval of at least 2 days, preferably from 3-9 days, most preferably from 5-8 days, during which no progestogenic component and no estrogenic component is administered and wherein the resulting decrease in serum concentration of the progestogenic component and the estrogenic component induces menses.

Yet another embodiment of the invention, which concerns a sequential method without a significant pause, is characterised in that it comprises the uninterrupted parenteral or rectal administration of the estrogenic component during a period of at least 28 days, preferably at least 60 days, and in that, following the combined administration of the estrogenic component and the progestogenic component, the estrogenic component and no progestogenic component are administered during 3-18 consecutive days, preferably during 5-16 consecutive days and

the resulting decrease in serum concentration of the progestogenic component should normally be sufficient to induce menses.

The mode of administration employed in the present method is suitably selected from the group consisting of transdermal, intranasal, intravaginal, rectal, pulmonary, buccal, subcutaneous, intramuscular or intra-uterine administration. In a particularly preferred embodiment the present method employs transdermal, intravaginal, intranasal or rectal administration. Even more preferably the present method employs transdermal or intranasal administration. The most preferred mode of administration is transdermal administration.

Rectal, intranasal, buccal and pulmonary administration are ideally suited for (at least) once daily administration. Transdermal administration is advantageously applied at frequencies between once a day and once a month. Intravaginal and intra-uterine administrations are advantageously operated at administration frequencies between once weekly and once monthly. Subcutaneous and intramuscular administration are suitably done in the form of depot injections at intervals of 1 week to 6 months, preferably at intervals of 4 weeks to 3 months.

For reasons of convenience and also to achieve high compliance rates, the present method preferably utilises administration intervals of 1 day, 1 week or 1 month. Regimens that employ once daily intranasal administration, once weekly transdermal administration or once monthly intravaginal or subcutaneous administration are particularly preferred.

Irrespective of the mode of administration, the estrogenic component is preferably administered in an amount effective to achieve a blood serum concentration of at least 1 nanogram per litre, more preferably of at least 10 nanogram per litre, most preferably at least 100 nanogram per litre. Generally the resulting blood serum concentration of the estrogenic component will not exceed 100 μg per litre, preferably it will not exceed 50 μg per litre, more preferably it will not exceed 25 μg per litre.

In accordance with the present method the estrogenic component is usually administered in an amount of less than 1 mg per kg of bodyweight per day, preferably of less than 0.4 mg per kg of bodyweight per day, more preferably of less than 0.2 mg per kg of bodyweight per day. In order to achieve a significant impact from the administration of the estrogenic component, it is advisable to administer in an amount of at least 1 μg per kg of bodyweight per day. Preferably, the administered amount is at least 2 μg per kg of bodyweight per day, more preferably at least 5 μg per kg of bodyweight per day. The aforementioned dosages are to be construed as averaged daily dosages in case administration intervals of more than 1 day are used.

In the present method, particularly when used in humans, the estrogenic component is usually administered parenterally or rectally in an average dosage of at least 0.05 mg per day, preferably of at least 0.1 mg per day. The average maximum parenteral or rectal dosage is normally kept below 40 mg per day, preferably below 20 mg per day.

In all of the aforementioned methods it is preferred to parenterally or rectally administer the estrogenic component and the progestogenic component during a period of at least 10, preferably of at least 20 days. In case of a sequential method without pause or a continuous combined method it is preferred to administer the estrogenic component and/or the progestogenic component uninterruptedly during a period of at least 30 days, more preferably of at least 60 days, most preferably of at least 150 days. Uninterrupted sequential HRT methods, which employ continuous estrogen administration, exhibit optimum cycle control. The general concerns about the so called unopposed administration of estrogen, i.e. administration of estrogen without co-administered progestogen might cause hyperplasia of the endometrium, are less applicable to the estrogenic components of the present invention. Therefore, in a particularly preferred embodiment, the present method is executed in accordance with a sequential HRT method without pause.

In the present methods the uninterrupted parenteral administration of the estrogenic component may usually occur at intervals of at least 12 hours, preferably of between 20 hours and 30 days. The relatively high *in vivo* halflife of the present estrogenic components in comparison to most known estrogens makes it feasible to employ administration intervals that are significantly longer than 1 day. With a view to compliance, however, it is preferred to employ once daily, once weekly or once monthly administration intervals. Naturally the length of the administration interval is largely determined by the mode of parenteral or rectal administration that is employed.

In accordance with the present invention the progestogenic component is advantageously administered in an amount which is effective to achieve a blood serum concentration which is equivalent to at least 5 pg/mL norethisterone, preferably to at least 10 pg/mL norethisterone. In the present method, blood serum concentrations of the progestogenic component will usually remain below the equivalent of 5,000 pg/mL norethisterone. Preferably these concentrations remain below the equivalent of 1,000 pg/mL.

In the present method the progestogenic component is usually administered in an amount of less than 1 mg per kg of bodyweight per day, preferably of less than 0.2 mg per kg of bodyweight per day. Furthermore, it is advisable to parenterally or rectally administer the progestogenic component in an amount of at least 0.1 μg per kg of bodyweight per day.

Preferably, the parenterally or rectally administered amount is at least 0.3 μg per kg of bodyweight per day. In humans, the progestogenic component is usually parenterally or rectally administered in an average dosage of at least 5 μg per day, preferably of at least 15 μg per day. The maximum dosages normally remain below 50 mg, preferably below 10 mg per day.

Examples of progestogens which may suitably be used in accordance with the present invention include: progesterone, levonorgestrel, norgestimate, norethisterone, dydrogesterone, drospirenone, 3-beta-hydroxydesogestrel, 3-keto desogestrel (=etonogestrel), 17-deacetyl norgestimate, 19-norprogesterone, acetoxypregnenolone, allylestrenol, anagestone, chlormadinone, cyproterone, demegestone, desogestrel, dienogest, dihydrogesterone, dimethisterone, ethisterone, ethynodiol diacetate, flurogestone acetate, gastrinon, gestodene, gestrinone, hydroxymethylprogesterone, hydroxyprogesterone, lynestrenol (=lynoestrenol), medrogestone, medroxyprogesterone, megestrol, melengestrol, nomegestrol, norethindrone (=norethisterone), norethynodrel, norgestrel (includes d-norgestrel and dl-norgestrel), norgestrienone, normethisterone, progesterone, quingestanol, (17alpha)-17-hydroxy-11-methylene-19-norpregna-4,15-diene-20-yn-3-one, tibolone, trimegestone, algestone acetophenide, nestorone, promegestone, 17-hydroxyprogesterone esters, 19-nor-17hydroxyprogesterone, 17alpha-ethinyl-testosterone, 17alpha-ethinyl-19-nor-testosterone, d-17beta-acetoxy-13beta-ethyl-17alpha-ethinyl-gon-4-en-3-one oxime and precursors of these compounds that are capable of liberating these progestogens *in vivo* when used in the present method. Preferably the progestogen used in the present method is selected from the group consisting of progesterone, desogestrel, etonogestrel, gestodene, dienogest, levonorgestrel, norgestimate, norethisterone, drospirenone, trimegestone, dydrogesterone, precursors of these progestogens and mixtures thereof.

The present method also encompasses the co-administration of active principles in addition to the progestogenic and estrogenic component. For instance, androgens may advantageously be co-administered in order to prevent symptoms of hypoandrogenicity. Thus, a preferred embodiment of the invention comprises the co-administration of an androgenic component. The androgenic component is suitably co-administered in an effective amount to suppress symptoms of hypoandrogenicity. Hypoandrogenicity has been associated with mood disturbances, unfavourable changes in haemostatic parameters and lack of bone mass.

The term "androgenic component" is defined as a substance that is capable of triggering an androgenic response *in vivo* or a precursor which is capable of liberating such a

substance *in vivo*. Usually androgenic components are capable of binding to an androgen receptor.

Androgenic components that may suitably be employed in the present method may be selected from the group consisting of testosterone esters such as testosterone undecanoate , testosterone propionate, testosterone phenylpropionate, testosterone isohexanoate, testosterone enantate, testosterone bucanate, testosterone decanoate, testosterone buciclate; testosterone; danazol; gestrinone; methyltestosterone; dehydroepiandrosterone (DHEA); DHEA-sulphate; mesterolon; stanozolol; androstenedione; dihydrotestosterone; androstanediol; metenolon; fluoxymesterone; oxymesterone; methandrostenolol; MENT; precursors capable of liberating these androgens when used in the present method and mixtures thereof. Preferably the testosterone esters employed in the present method comprise an acyl group which comprises at least 6, more preferably from 8-20 and preferably 9-13 carbon atoms. Androgens that can be used advantageously in the present method include testosterone esters, testosterone and MENT. Most preferably the employed androgen is testosterone undecanoate.

In order to obtain the desired impact from the present method it is advisable to administer doses in an amount which leads to an increase in blood serum androgen level of at least 0.1 nmole testosterone equivalent per litre, preferably of at least 0.3 nmole testosterone equivalent per litre. Generally the method leads to an increase in blood serum androgen level of no more than 5 nmole testosterone equivalent per litre, preferably of less than 3 nmole testosterone equivalent per litre and most preferably of less than 1.5 nmole testosterone equivalent per litre.

The present method preferably does not employ a gonadotropin hormone releasing hormone composition as described in the aforementioned patents US 5,211,952, US 5,340,584 and US 5,340,585. Similarly, the present method preferably does not employ a luteinizing hormone releasing hormone composition as described in US 4,762,717 and US 5,130,137. Furthermore, the present method preferably does not comprise the co-administration of an anti-progestogen as described in US 5,468,736. The method may also suitably be applied without the co-administration of an antisense oligonucleotide that is complementary to the nucleotide sequence of the follicle stimulating hormone (FSH) receptor (WO 00/73416).

The present method is not suitable for oophorectomised females or for females in whom endometrial stimulation by estrogenic compositions is minimised or absent, e.g. as a result of hysterectomy.

Another aspect of the invention relates to a drug delivery system for parenteral or rectal administration that contains the estrogenic component as defined herein before and optionally the progestogenic component as described herein before, which drug delivery system is selected from the group consisting of suppositories, systems for intravaginal delivery, injectable or implantable depot preparations, inhalers, nasal sprays and transdermal delivery systems, wherein the system contains at least 0.01 mg, preferably at least 0.05 mg of the estrogenic component, and an androgenic component in an of at least 25 μg, preferably of at least 100 μg.

In accordance with the present invention, the drug delivery system containing the estrogenic component and the androgenic component may be administered separately from the progestogenic component, in particular if said progestogenic component is adminstered orally. Preferably, however, the system additionally contains a progestogenic component in an amount of at least 10 μg, more preferably of at least 30 μg of a progestogenic component.

In the present kit, the progestogenic component may conveniently be combined with the estrogenic component in a single parenteral or rectal dosage unit, e.g. a single transdermal patch, intravaginal ring, suppository or injection unit.

Transdermal delivery systems include patches, gels, tapes and creams, and can contain excipients such as solubilisers, permeation enhancers (e.g. fatty acids, fatty acid esters, fatty alcohols and amino acids), hydrophilic polymers (e.g. polycarbophil and polyvinyl pyrrolidine) and adhesives and tackifiers (e.g. polyisobutylenes, silicone-based adhesives, acrylates and polybutene).

Transmucosal delivery systems include patches, suppositories, pessaries, gels, and creams, and can contain excipients such as solubilizers and enhancers (e.g. propylene glycol, bile salts and amino acids), and other vehicles (e.g. polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethyl cellulose and hyaluronic acid).

Injectable depot systems include solutions, suspensions, gels, microspheres and polymeric injectables, and can comprise excipients such as solubility-altering agents (e.g. ethanol, propylene glycol and sucrose) and polymers (e.g. polycaprylactones, and PLGA's). Implantable depot systems include rods and discs, and can contain excipients such as PLGA and polycapryl lactone. Suitable fluid carrier components are physiologically compatible diluents wherein the active agents can be dissolved, suspended. An example of a diluent is water, with or without addition of electrolyte salts or thickeners. Thus, the depot formulation can be, for example, an aqueous microcrystalline suspension. Oils are particularly suitable as

diluents, with or without the addition of a solubiliser, of a surfactant, or of a suspension or emulsifying agent. Examples of suitable oils include arachidis oil, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil, and sesame oil. Examples of solubilisers include benzyl alcohol and benzyl benzoate. Depot preparations offer the advantage that a single injection or implantation suffices for one or several months. Duration of the depot effect depends the nature of the estrogenic component (the ester precursors being preferred as they display a slower release), the amount of the estrogenic component as well as on the type of carrier substance that releases the active agent. Generally, the duration will be in the range of 10-30 days, but longer or shorter times can also be achieved.

Other delivery systems that can be used for administering the pharmaceutical composition of the invention include intranasal and pulmonary delivery systems such as sprays and microparticles.

The present invention is further illustrated by the following examples, which, however, are not to be construed as limiting. The features disclosed in the foregoing description, in the following examples and in the claims may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

EXAMPLES

Example 1

Vaginal cornification was chosen as a tissue-specific and estrogen-sensitive endpoint to determine the estrogenicity of estetrol (E4), after subcutaneous administration, in hypoestrogenic rats. 17β-estradiol (E2) and vehicle (10% ethanol/sesame oil) served as controls in the bioassay.

Uterine weight increase in the rat is more commonly used as a measure of estrogenicity. However, uterine weight also responds to progesterone, testosterone, and other agents not characteristically regarded as estrogens. In the early 1920s it was discovered that follicular fluid from the pig ovary contained a factor(s) that caused cornification/keratinization of the vaginal epithelium in the rat (Allen and Doisy, 1923, JAMA, 81, 819-821; Allen and Doisy, 1924, Am. J. Physiol., 69, 577-588). The so-called vaginal cornification response in rats subsequently provided a bioassay for testing estrogenicity. Vaginal epithelial cornification/keratinization in ovariectomized rats can be produced only by compounds considered to be true estrogens (Jones et al, 1973, Fert. Steril.

24, 284-291). Vaginal epithelial cornification/keratinization represents, therefore, a highly selective endpoint to determine the potency of estrogens (Reel et al., 1996, Fund. Appli. Toxicol. 34, 288-305).

Adult intact female CD rats were ovariectomized to induce estrogen deficiency. Vaginal lavages were performed daily for seven days to ensure that the rats demonstrated castrate vaginal smears (predominance of leukocytes in the vaginal smear, and similar in appearance to a diestrous vaginal smear). Castrate vaginal smears are indicative that complete ovariectomy was achieved. Treatment commenced following completion of the 7 days of smearing (day 0 = first day of dosing). Animals were dosed, once daily for 7 consecutive days. Daily vaginal lavages continued to be obtained for 7 days after dosing was initiated in order to detect vaginal cornification, as an indication of an estrogenic response. A drop of vaginal washings was placed on a glass slide and examined by light microscopy to detect the presence or absence of cornified epithelial cells. Vaginal lavages were obtained prior to dosing on days 0-6 and prior to necropsy on day 7.

The vaginal cornification bioassay was performed in order to determine the estrogenic profile of E4 when given subcutaneously (sc) to ovariectomized adult rats. E2 was used as a positive control. The vehicle (10% ethanol/sesame oil) served as the negative control. Steroids were dissolved in absolute ethanol and then brought to the final concentration with sesame oil (10% ethanol in sesame oil). The occurrence of vaginal cornification, indicative of an estrogenic response, is an "all or none" response. Data are, therefore, expressed as the number of rats showing a vaginal estrogenic response over the number of rats (ratio) treated.

A vaginal estrogenic response occurred in 8/8 rats by day 2 and persisted through day 7 in rats injected sc with 50 µg/kg/day E2 for 7 days (Table 1). Animals treated with the vehicle did not exhibit vaginal epithelial cornification (Table 1). The onset of vaginal epithelial cornification was dose-dependent in rats injected sc with 0.1, 0.3, 1.0, and 3.0 mg/kg/day E4 and started at the same day of treatment (Day 2) as observed for E2 (Table 1). At 0.1 mg/kg/day E4 already 4/8 rats and at 0.3 mg/kg/day E4 even 7/8 rats exhibited a vaginal estrogenic response by day 7. At 1.0 and 3.0 mg/kg/day E4 all rats showed a vaginal estrogenic response by day 7 (Table 1).

Table 1: Vaginal estrogenic response in ovariectomized rats treated subcutaneously (sc) with 17β-estradiol (E2) or estetrol (E4). Data are expressed as the number of rats showing vaginal cornification over the number of rats (ratio) treated.

Treatment Group	Dosing route	Number of Rats Exhibiting Estrogenic Response/ Number of Rats Treated							
		Day of Study							
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.05 mg/kg/day E2	sc	0/8	0/8	8/8	8/8	8/8	8/8	8/8	8/8
Vehicle Control	sc	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
0.1 mg/kg/day E4	sc	0/8	0/8	0/8	1/8	1/8	4/8	3/8	4/8
0.3 mg/kg/day E4	sc	0/8	0/8	1/8	5/8	7/8	6/8	7/8	7/8
1.0 mg/kg/day E4	sc	0/8	0/8	1/8	6/8	8/8	7/8	8/8	8/8
3.0 mg/kg/day E4	sc	0/8	0/8	3/8	8/8	8/8	8/8	8/8	8/8

Example 2

To determine the elimination half-life of estetrol (E4) after subcutaneous administration (sc), single dose studies were performed in female Sprague Dawley rats followed by frequent blood sampling over a 24 hours interval.

Female Sprague Dawley rats were equipped with a permanent silatic heart catheter, as described by Kuipers et al. (1985, Gastroenterology, 88, 403-411). Rats were allowed to recover from surgery for 5 days and were than administered 0.05, 0.5, or 5 mg/kg E4 in 0.5 ml arachidis oil. E4 was injected in the neck area using a 1 ml syringe and 20g needle. Blood samples were subsequently collected via the heart catheter in heparinized tubes at 0.5, 1, 2, 4, 8 and 24 hours. Erythrocytes were removed by centrifugation at 5000xg for 10 minutes at 4°C and blood plasma was stored at –20°C. After thawing the plasma samples, liquid-liquid extraction (hexane and diethyl ether) was employed to prepare the E4-containing plasma samples for HPLC analysis (Perkin Elmer 200) and tandem mass spectrometry using a PE Sciex 3000 tandem mass spectrometer and APCI interface. With each sample batch, a calibration curve with 6 calibrators was recorded. The calibration curve was calculated using

linear regression (correlation coefficient > 0.98), which permitted quantitation of plasma concentrations. For each rat plasma, sampled at different time intervals, data were collected.

Plasma E4 concentration data were analysed with "WinNonLin, edition 3.1" and involved pharmacokinetic parameters for C_{max}, AUC_{0-24} and half-life. Interestingly, E4 demonstrated a relatively long half-life of 2-3 hours, enabling the detection of bioactive levels of unconjugated E4 at all time points over a 24 hour interval.

Example 3

An established competitive steroid-binding assay (Hammond and Lahteenmaki. 1983. Clin Chem Acta 132:101-110) was used to determine the relative binding affinity of estetrol (E4), 17α-ethinylestradiol(EE2), 17β-estradiol (E2), testosterone (T)and 5α-dihydrotestosterone (DHT) for human sex Hormone Binding Globulin (SHBG).

Human SHBG was purified from transgenic mouse serum, as described previously (Avvakumov GV et al., 2000. J Biol Chem 275: 25920-25925). The human SHBG prepared in this way was assessed to be >99% pure by polyacrylamide gel electrophoresis under denaturing conditions. Its steroid-binding characteristics are indistinguishable from SHBG in human serum (Avvakumov GV et al., 2000. J Biol Chem 275: 25920-25925). The *in vitro* assay involved the use of the purified human SHBG and [^{3}H]DHT or [^{3}H]estradiol as labeled ligands. Human SHBG was treated for 30 min at room temperature with a dextran-coated charcoal (DCC) suspension in phosphate buffered saline (PBS) to remove any steroid ligand. After centrifugation (2,000 x g for 10 min) to sediment the DCC, the supernatant containing the human SHBG was diluted in PBS to a concentration of 1 nM based on its steroid binding capacity.

Duplicate aliquots (100 µl) of this human SHBG solution were then incubated with an equal volume of either [^{3}H]DHT or [^{3}H]estradiol at 10 nM, together with 100 µl of PBS alone or the same amount of PBS containing increasing concentrations of unlabeled steroid ligands as competitors in polystyrene test tubes. After incubation for 1 h at room temperature the reaction mixtures were placed in an ice bath for a further 15 min. Aliquots (600 µl) of an ice cold suspension of DCC were then added to each tube, and after a brief 2 seconds mixing, each tube was incubated in an ice bath for either 10 min or 5 min depending on whether [^{3}H]DHT or [^{3}H]estradiol were being used as labeled ligands, respectively. The unbound ligands adsorbed to DCC were then removed by centrifugation (2, 000 x g for15 min at 4 C), and the amounts of [^{3}H]labeled ligands bound to SHBG were counted in 2 ml ACS scintillation cocktail using in liquid scintillation spectrophotometer. The average amounts of

IOCID: <WO__03018026A1_I_>

[^{3}H]labeled ligands bound to SHBG at each concentration of competitor (B) were expressed as a percentage of the average amounts of [^{3}H]labeled ligands bound to SHBG in the absence of competitor (B_0), and were plotted against the concentration of competitor in each assay tube. The results of the competitive binding assays are depicted in Figure 1.

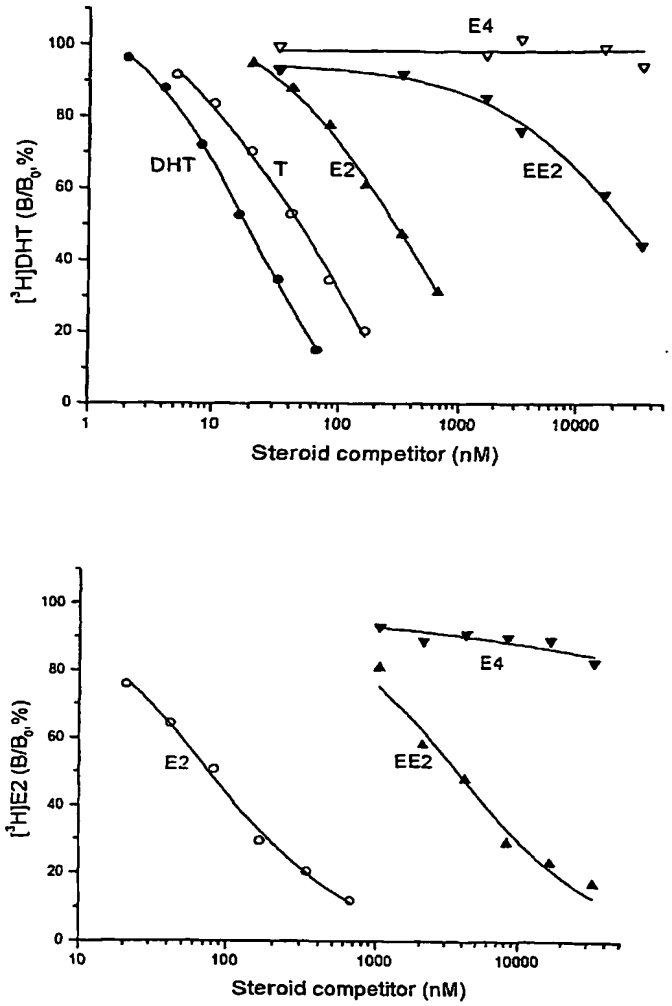


Figure 1: Competitive displacement of [^{3}H]DHT (panel A) and [^{3}H]estradiol (panel B) from the human sex hormone-binding globulin steroid binding site. The unlabeled steroid ligands used as competitors were as follows: estetrol (E4), 17α-ethinylestradiol (EE2), 17β-estradiol (E2), testosterone (T) and 5α-dihydrotestosterone (DHT)

As is clearly apparent from these competitive binding assays, estetrol does not bind at all to human SHBG when tested with either [^{3}H]DHT or [^{3}H]estradiol as labeled ligands. This is in marked contrast with reference steroids ethinylestradiol, 17β-estradiol, testosterone and 5α-dihydrotestosterone, which, in this order, show an increased relative binding affinity for

human SHBG. Importantly, estetrol binding to SHBG was negligible when compared with the other estrogens tested, ethinylestradiol and 17β-estradiol.

Example 4

The ovariectomized aged rat is used as a model for the human disease osteoporosis. This is an established animal model, recommended by the United States Food and Drug Administration (FDA), to evaluate and assess potential agents for osteoporosis prevention and therapy. The anti-resorptive efficacy of estetrol (E4) after subcutaneous administration (sc) is tested by *ex vivo* measuring total and trabecular bone mineral density and bone strength after 4 weeks of treatment at necropsy. 17α-ethinyl-estradiol (EE), 17β-estradiol (E2) and vehicle (1 % ethanol/arachidis oil) serve as controls in this bioassay.

Three months old female Sprague-Dawley rats (10 per treatment group) are either sham-operated (Sham) or ovariectomized (OVX) one day prior to the start of sc test compound dosing with vehicle, EE (0.3, 1.0, 3.0, 30, or 100 μg/kg/day), E2 (0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg/day) or E4 (0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg/day). After 4 weeks of treatment, at necropsy, tibiae and femura are removed, cleaned of soft-tissue and fixed and stored in 70% ethanol/saline at 4 °C (tibia) or saline at 4 °C (femura) until further analysis. Susequently, *ex vivo* peripheral Quantitative Computed Tomography (pQCT) is performed on the excised tibiae using a Stratec XCT-RM and associated software (Stratec Medizintechnik GmbH, Pforzheim, Germany, software version 5.40). The scans are performed at 12 % of the total length from the proximal end of the tibiae and the mineral content, area and density for total and trabecular bone are determined. Furthermore, *ex vivo* evaluation of bone biomechanical strength is performed with an indentation test at the distal femura. Prior to mechanical testing femura are rinsed in cold saline and are carefully cleaned of any remaining adherent soft tissue. A 3-mm segment of the distal femoral metaphysis is cut directly proximal to the femoral condyle with a low-speed diamond saw under constant saline irrigation. The load is applied with a cylindrical indenter (with a flat testing face of 1.6 mm diameter) to the center of the marrow cavity on the distal face of the segment. The indenter is allowed to penetrate the cavity at a constant displacement of 6 mm/min to a depth of 2 mm before load reversal. The maximum load, stiffness and energy absorbed are determined from load displacement curves. Ultimate strength is calculated by dividing the maximum load by the indenter area.

By comparison of the pQCT densitometry data from the proximal tibiae of Sham-operated and OVX-rats it is clearly shown that ovariectomy results in a consistent loss of total and trabecular bone. Four 4 weeks sc treatment with either EE, E2 or E4 are found to dose-dependently inhibit bone resorption when total and trabecular bone density are monitored as endpoints. Similar to both EE and E2, complete prevention of bone loss and preservation of bone strength are achieved with sc treatment of E4. By sc E4 administration, bone strength can be fully protected to levels of sham-operated rats.

Example 5

The morphine-dependent ovariectomized (OVX) rat is used as a model for postmenopausal hot flush. The potency of estetrol (E4) to prevent tail skin temperature rises, normally accompanied by a drop in core body temparature, after naloxone-induced opiate withdrawel is tested, after subcutaneous administration (sc) of E4. 17α-ethinyl-estradiol (EE) and vehicle (hydroxy propyl-beta-cyclodextrin 20% wt/vol) serve as controls in this bioassay.

The most common and characteristic symptom of human menopause is the hot flush, which is experienced by over 70% of menopausal females. While the exact mechanism underlying this vasomotor instability is unknown, the characteristic features of the hot flush appear to reflect a centrally mediated adaptation to a progressive decline in the levels of estrogens. In women experiencing the hot flush the symptoms are manifested by 1) rapid, regional elevations in skin temperature; 2) a decrease in core body temperature; 3) an increased heart rate with no change in blood pressure; and 4) closely timed surges in release of luteinizing hormone (LH) and β-endorphin.

The morphine-dependent ovariectomized (OVX) rat model has been proposed by several investigators (Katovich et al, 1986, Maturitas, 67-76; Merchenthaler et al. 1998, Maturitas, 307-316) as an animal model for the hot flush. During opiate withdrawal with the morphine antagonist naloxone, tail skin temperature (TST) rises and this rise is accompanied by a drop in core body temperature. In addition, the temperature changes are accompanied by surges in LH and a transient tachycardia. These events are similar in magnitude and temporal nature to those observed in the menopausal hot flush.

8-week-old OVX rats (6 animals per group) are sc treated with vehicle, EE (0.3 mg/kg/day) or E4 (0.1, 0.3, 1.0, or 3.0 mg/kg/day) for seven consecutive days prior to, and on the morning of naloxone-induced opiate withdrawal in morphine-dependent animals. Three days prior to the commencement of dosing, animals are anesthetized using a ketamine/xylazine anesthetic mixture and undergo a bilateral ovariectomy. Morphine

dependency is induced by implantation of subcutaneous pellets containing 75-mg morphine. The first pellet is implanted five days before the hot flush session under a light inhalation anesthesia. Three days before the hot flush session, two additional morphine pellets are implanted under the same conditions. For the hot flush manipulations, the animals are placed in a test cage. Following a 5 – 10 minute adaptation period, the rats are anesthetized with ketamine HCl 10 minutes prior to the hot flush session. A temperature sensitive electrode is fixed to the ventral surface of the tail with tape and the electrode is connected to a multi-channel temperature recorder. The tail-skin temperature is recorded until it is stable and the animals are then injected with naloxone HCl (1 mg/kg). The temperature recordings are continued for a period of 60 minutes and the temperature. At the completion of the hot flush session, all animals are killed by CO_2 asphyxiation followed by cervical dislocation.

Treatment with vehicle is not effective in preventing the naloxone-induced TST increases in the morphine addicted OVX rats. EE, at the single dose tested of 0.3 mg/kg/day, prevents the naloxone-induced TST increase in the morphine addicted OVX rats. Subcutaneous treatment with E4 shows a clear dose-dependent effect in preventing the naloxone-induced TST increase in the morphine addicted OVX rats to a similar degree as the potent estrogen (EE).

Example 6

Suitable formulations for the transdermal administration of estrogens are known in the art, and may be employed in the methods of the present invention. For example, suitable transdermal patch formulations for the administration of exogenous estrogen are described in US 4,460,372 (Campbell et al.), US 4,573,996 (Kwiatek et al.), US 4,624,665 (Nuwayser), US 4,722,941 (Eckert et al.), US 5,223,261 (Nelson et al.), the disclosures of which are hereby incorporated by reference.

One suitable type of transdermal patch for use in the methods of the present invention includes a backing layer which is non-permeable, a permeable surface layer, an adhesive layer substantially continuously coating the permeable surface layer, and a reservoir located or sandwiched between the backing layer and the permeable surface layer such that the backing layer extends around the sides of the reservoir and is joined to the permeable surface layer at the edges of the permeable surface layer. The reservoir contains the estrogenic component and is in fluid contact with the permeable surface layer. The transdermal patch is adhered to the skin by the adhesive layer on the permeable surface layer, such that the permeable surface

layer is in substantially continuous contact with the skin when the transdermal patch is adhered to the skin.

While the transdermal patch is adhered to the skin of the subject, the estrogenic component contained in the reservoir of the transdermal patch is transferred via the permeable surface layer, through the adhesive layer, and to and through the skin of the subject. The transdermal patch may suitably include one or more penetration-enhancing agents in the reservoir that enhance the penetration of the estrogenic component through the skin.

Examples of suitable materials which may comprise the backing layer are well known in the art of transdermal patch delivery, and any conventional backing layer material may be employed in the transdermal patch of the instant invention. Specific examples of suitable backing layer materials include but are not limited to polyester film, such as high density polyethylene, low density polyethylene or composites of polyethylene; polypropylene; polyvinyl chloride, polyvinylidene chloride; ethylene-vinyl acetate copolymers; and the like.

Examples of suitable permeable surface layer materials are also well known in the art of transdermal patch delivery, and any conventional material which is permeable to the estrogenic component, may be employed in the transdermal patch of the instant invention. Specific examples of suitable materials for the permeable surface layer include but are not limited to dense or microporous polymer films such as those comprised of polycarbonates, polyvinyl chlorides, polyamides, modacrylic copolymers, polysulfones, halogenated polymers, polychloroethers, acetal polymers, acrylic resins, and the like. Specific examples of these types of conventional permeable membrane materials are described in U.S. Pat. No. 3,797,494 to Zaffaroni.

Examples of suitable adhesives which may be coated on the backing layer to provide the adhesive layer are also well known in the art and include, for example pressure sensitive adhesives such as those comprising acrylic and/or methacrylic polymers. Specific examples of suitable adhesives include polymers of esters of acrylic or methacrylic acid (e.g., n-butanol, n-pentanol, isopentanol, 2-methyl butanol, 1-methyl butanol, 1-methyl pentanol, 3-methyl pentanol, 3-methyl pentanol, 3-ethyl butanol, isooctanol, n-decanol, or n-dodecanol esters thereof) alone or copolymerized with ethylenically unsaturated monomers such as acrylic acid, methacrylic acid, acrylamide, methacrylamide, N-alkoxymethyl acrylamides, N-alkoxymethyl methacrylamides, N-t-butylacrylamide, itaconic acid, vinyl acetate, N-branched C.sub.10-24 alkyl maleamic acids, glycol diacrylate, or mixtures of the foregoing; natural or synthetic rubbers such as silicon rubber, styrene-butadiene rubber, butyl-ether rubber, neoprene rubber, nitrile rubber, polyisobutylene, polybutadiene, and polyisoprene;

polyurethane elastomers; vinyl polymers such as polyvinyl alcohol, polyvinyl ethers, polyvinyl pyrrolidone, and polyvinyl acetate; ureaformaldehyde resins; phenol formaldehyde resins; resorcinol formaldehyde resins; cellulose derivatives such as ethyl cellulose, methyl cellulose, nitrocellulose, cellulose acetatebutyrate, and carboxymethyl cellulose; and natural gums such as guar, acacia, pectin, starch, destria, gelatin, casein, etc.

As will be apparent to those skilled in the art, the adhesive layer should be inert to the estrogenic component, and should not interfere with the transdermal delivery of the estrogenic component through the permeable surface layer. Pressure sensitive adhesives are preferred for the adhesive layer of the transdermal patch to facilitate the application of the patch to the skin of the subject.

Suitable penetration-enhancing agents are well known in the art as well. Examples of conventional penetration-enhancing agents include alkanols such as ethanol, hexanol, cyclohexanol, and the like; hydrocarbons such as hexane, cyclohexane, isopropylbenzene; aldehydes and ketones such as cyclohexanone, acetamide; N,N-di(lower alkyl)acetamides such as N,N-diethylacetamide, N,N-dimethyl acetamide,; N-(2-hydroxyethyl)acetamide; esters such as N,N-di-lower alkyl sulfoxides; essential oils such as propylene glycol, glycerine, glycerol monolaurate, isopropyl myristate, and ethyl oleate; salicylates; and mixtures of any of the above.

In another example of a transdermal patch which is suitable for the transdermal delivery of the estrogenic component according to the present invention, said estrogenic component is incorporated into the adhesive layer rather than being contained in a reservoir. Examples of these types of patches are conventionally known and include, for example, the CLIMERA.®. patch available from Berlex. This type of transdermal patch comprises a backing layer and an adhesive/drug layer. The adhesive/drug layer has the combined function of adhering the patch to the skin of the subject and containing the estrogenic component, which is to be administered. The active ingredient is leached from the adhesive/drug layer to and through the skin of the subject when the patch is adhered to the skin.

Any of the backing layers described herein above may be employed in this embodiment as well. In addition, any of the suitable adhesives described above may be employed. The adhesive/drug layer comprises a relatively homogeneous mixture of the selected adhesive and the active ingredient. Typically, the adhesive/drug layer comprises a coating substantially covering one surface of the backing layer. The adhesive/drug layer may also include a penetration enhancing agent such as those described above by incorporating the

penetration enhancing agent into the substantially homogeneous mixture of the adhesive and the active ingredient.

As will be readily apparent to those skilled in the art, the transdermal patches according to the present invention may include a variety of additional excipients which are conventionally employed to facilitate the transdermal administration of the estrogenic component. Examples of such excipients include but are not limited to carriers, gelling agents, suspending agents, dispersing agents, preservatives, stabilisers, wetting agents, emulsifying agents, and the like. Specific examples of each of these types of excipients are well known in the art and any conventional excipients may be employed in the transdermal patches of the instant invention.

The amount of estrogenic component contained in the transdermal patch formulations will depend upon the precise form of estrogenic component to be administered, but should be sufficient to deliver at least 20 μg per day. The amount of progestogenic component to be administered is typically equivalent to an amount of at least 100 μg norethisterone acetate per day. Typically, the transdermal patches are designed to be worn for several days before replacement is required. Thus the amount of estrogenic component in the patch must be sufficient to permit the administration of at least 20 μg per day for a period of several days. As an example, a transdermal patch according to the present invention which is designed to administer around 400 μg of estetrol and 250 μg norethisterone acetate per day for seven (7) days would contain approximately 40 mg of the estrogen and approximately 25 mg of the progestogen. Based upon this information, one skilled in the art would be able to establish the necessary amount of estrogenic component to be included in a given transdermal patch to achieve the delivery of the correct daily dose of estrogenic component.

Example 7

Suitable nontoxic pharmaceutically acceptable carriers for use in a drug delivery system for intranasal administration of the present estogenic component will be apparent to those skilled in the art of nasal pharmaceutical formulations. For those not skilled in the art, reference is made to "Remington's Pharmaceutical Sciences", 4th edition, 1970. Obviously, the choice of suitable carriers will depend on the exact nature of the particular nasal dosage form desired, e.g. whether the estrogenic component is to be formulated into a nasal solution (for use as drops or as a spray), nasal microspheres, a nasal suspension, a nasal ointment or a nasal gel, as well as on the identity of the estrogenic component.

Examples of the preparation of typical nasal compositions are set forth below.

XOCID: <WO_03018026A1_I_>

Nasal solution:

15 mg of estetrol and 15 mg of progesterone are combined with 10 mg of Tween 80. That mixture is then combined with a quantity of isotonic saline sufficient to bring the total volume to 50 ml. The solution is sterilised by being passed through a 0.2 micron Millipore filter.

Nasal gel:

250 ml of isotonic saline are heated to 80°C. and 1.5 g of Methocel are added, with stirring. The resultant mixture is allowed to stand at room temperature for 2 hours. Then, 25 mg of estetrol and 25 mg of progesterone are mixed together with 10 mg of Tween 80. The estetrol/Tween mixture and a quantity of isotonic saline sufficient to bring the total volume to 500 ml were added to the gel and thoroughly mixed.

Example 8

The intravaginal drug delivery vehicle may suitably take the form of a vaginal ring. Vaginal rings are torous shaped devices designed to deliver a relatively constant dose of drug to the vagina usually over a period of weeks to months. Typically, they are made of a poly EVA elastomer and the estrogenic component is released by diffusion though the elastomer. The vaginal ring is designed to regulate the release rate of the estrogenic component so as to provide the user with the appropriate daily dose. Among the important factors governing release are the solubility of the estrogenic component in the ring elastomer, the surface area of the drug reservoir, the distance the drug must diffuse through the ring body to reach its surface and the molecular weight of the drug.

If relatively high release rates are desired, they can be attained by a drug load at the ring surface as is characteristic of the homogeneous matrix ring design. This design, however, suffers from rapidly declining release rates as the distance the drug must travel to reach the ring surface increases as the drug load near the surface is depleted. If moderately high release rates are needed to provide the appropriate dose, a design which modulates release rate by imposing a layer of drug-free elastomer between the drug reservoir and the ring exterior is appropriate. This may be attained by coating a homogeneous ring, or to conserve drug, by incorporating a drug-free core, a shell design may be used. If an even lower release rate is desired, the drug may be confined to a small diameter at the center of the ring ("core ring").

Numerous types of vaginal rings have been described in the patent and non-patent literature alike.

An example of the preparation of an estetrol containing intravaginal ring is set forth below:

Four 58 mm core rings are prepared as follows. Fifty grams of Silastic 382 ® are mixed with 0.3 g of stannous octoate, transferred to a 50 cc plastic syringe and injected into four brass ring moulds. After 45 minutes, the moulds are opened, the rings removed, the flash is trimmed and the rings are cut open at a 45° angle. A mixture of 84.4 g Silastic 382®, 24.4 g of micronised estetrol and 12.2 g micronised levonorgestrel are mixed in a Teflon bowl. The mixture is transferred to a Lucite coating cup with a bottom opening of 8.7 mm. The open rings are heated at 110°C for 30 minutes, cooled and weighed. The open rings weigh approximately 9.8 g. The open rings are pulled through the coating cup and dipped in a solution of 0.67% stannous octoate in toluene (w/v). The open ring is again heated at 110°C for 30 minutes and reweighed. The weight of the coated open ring is approximately 10.3 g and the weight of the coating on the open rings is therefore approximately 0.5 g.

In order to apply the outer layer a 16.5 cm long piece of silicone rubber tubing having 6.3 mm diameter and 0.3 mm wall thickness is swollen in hexane and the open ring coated with the medicated layer is placed inside the silicone rubber tubing. The hexane is evaporated at room temperature and the tubing contracted to the size of the open ring forming an outer layer having a thickness of 0.2 mm.

The excess tubing is trimmed flush with the ends of the open ring and Dow Corning Medical Adhesive A is applied at both ends of the open ring and to 1 cm of the outer layer at both ends of the open ring. A 4 cm piece of silicone tubing 6.3 mm inner diameter and 0.3 mm wall thickness is swollen with hexane and placed over the two ends of the open ring to close the ring. The ring is held for about two minutes until the tubing has shrunk and fits snugly over the ring junction. The adhesive is allowed to cure for 24 hours, the rings are rinsed in alcohol and air dried.

Example 9

An estetrol containing depot formulation can suitably be prepared as set forth below.

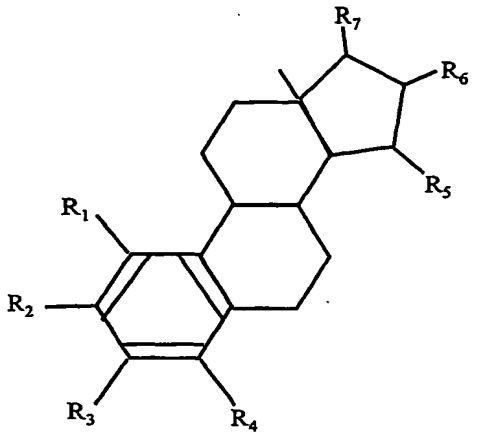
At room temperature, 2000 mg estetrol, 500 mg are dispersed in 6 millilitre dehydrated ethanol. This solution is then diluted with 660 ml arachidis oil under thorough

stirring and the addition of 1000 mg testosterone undecanoate. The resulting solution is sterilised by filtration.

In case an estetrol ester is used, e.g. estetrol valerate esters, a significantly lower release rate can be obtained. Such low release rates are particularly advantage if the depot injections are to be administered at relatively long time intervals, e.g. intervals of more than 1 week.

CLAIMS

1. Use of an estrogenic component selected from the group consisting of:
substances represented by the following formula



in which formula R_1, R_2, R_3, R_4 independently are a hydrogen atom, a hydroxyl group or an alkoxy group with 1-5 carbon atoms; each of R_5, R_6, R_7 is a hydroxyl group; and no more than 3 of R_1, R_2, R_3, R_4 are hydrogen atoms;
precursors capable of liberating a substance according to the aforementioned formula when used in the present method; and
mixtures of one or more of the aforementioned substances and/or precursors;
in the manufacture of a pharmaceutical composition for use in a method of hormone replacement in mammals, which method comprises the parenteral or rectal administration of said estrogenic component and a progestogenic component to a mammal in an effective amount to treat or prevent symptoms of hypoestrogenism.

2. Use according to claim 1, wherein the symptoms of hypoestrogenism are selected from the group consisting of osteoporosis, arteriosclerosis, climacteric symptoms, cognitive disturbances and Alzheimer's disease.

3. Use according to claim 1 or 2, wherein R_3 represents a hydroxyl group or an alkoxy group.

4. Use according to any one of claims 1-3, wherein at least 3 of the groups R_1, R_2, R_3 and R_4 represent hydrogen atoms.

5. Use according to any one of claims 1-4, wherein the precursors are derivatives of the substances represented by the formula of claim 1, wherein the hydrogen atom of at least one of the hydroxyl groups in said formula has been substituted by an acyl radical of a hydrocarbon carboxylic, sulfonic or sulfamic acid of 1-25 carbon atoms; tetrahydrofuranyl; tetrahydropyranal; or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue.

6. Use according to any one of claims 1-5, wherein the method comprises the uninterrupted administration of the estrogenic component during a period of at least 10 days, preferably of at least 20 days.

7. Use according to claim 6, wherein the method comprises the uninterrupted administration, during a period of at least 10 days, of a combination of the estrogenic component and a progestogenic component.

8. Use according to claim 7, wherein the method comprises the uninterrupted administration of the combination of the estrogenic component and the progestogenic component during a period of at least 28, preferably at least 60 days.

9. Use according to claim 7, wherein the method comprises an interval of at least 2 days, preferably of 3-9 days, during which no progestogenic component and no estrogenic component is administered and wherein the resulting decrease in serum concentration of the progestogenic component and the estrogenic component induces menses.

10. Use according to claim 7, wherein the method comprises the uninterrupted administration of the estrogenic component during a period of at least 28 days, preferably at least 60 days, and wherein, following the combined administration of the estrogenic component and the progestogenic component, the estrogenic component and no progestogenic component are administered during 3-18 consecutive days and the resulting decrease in serum concentration of the progestogenic component induces menses.

11. Use according to any one of claims 1-10, wherein the method comprises the transdermal, intranasal, intravaginal, rectal, pulmonary, buccal, subcutaneous or intra-uterine administration of the estrogenic component and the progestogenic component.

12. Use according to any one of claims 1-11, wherein the estrogenic component is administered in an amount effective to achieve a blood serum concentration of at least 0.02 μg, preferably of at least 0.1 μg per litre.

13. Use according to any one of claims 1-12, wherein the estrogenic component is administered in an amount of at least 1 μg per kg of bodyweight per day, preferably of at least 5 μg per kg of bodyweight per day.

14. Use according to any one of claims 1-13, wherein the progestogenic component is administered in an amount effective to achieve a blood serum concentration which is equivalent to at least 5 pg/mL norethisterone, preferably to at least 10 pg/mL norethisterone.

15. A drug delivery system for parenteral or rectal administration that contains an estrogenic component, said drug delivery system being selected from the group consisting of suppositories, systems for intravaginal delivery, injectable or implantable depot preparations, inhalers, nasal sprays and transdermal delivery systems, wherein the system contains at least 0.01 mg, preferably at least 0.05 mg of the estrogenic component as defined in claim 1 and at least 25 μg of an androgenic component.

16. Drug delivery system according to claim 15, wherein the device additionally contains at least 10 μg, preferably at least 30 μg of a progestogenic component.

17. Drug delivery system according to claim 15 or 16, wherein the androgenic component is selected from the group consisting of testosterone esters; testosterone; danazol; gestrinone; methyltestosterone; dehydroepiandrosterone (DHEA); DHEA-sulphate; mesterolon; stanozolol; androstenedione; dihydrotestosterone; androstanediol; metenolon; fluoxymesterone; oxymesterone; methandrostenolol; MENT; precursors capable of liberating these androgens when used in the present method and mixtures thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/ 02/00333

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/565 A61K31/57 A61P5/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 827 843 A (KONINCKX PHILIPPE ROBERT MARIE) 27 October 1998 (1998-10-27) column 1, line 8-20 column 2, line 65-67 claims 1,5,6,9 ---	1-14
Y	EP 0 136 011 A (PLUNKETT EARL ROBERT ;WOLFE BERNARD M J (GB)) 3 April 1985 (1985-04-03) tables 1A,1B page 10, line 6,7 claims 1,3,4,6 --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) .

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2002	0 [illegible]

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 340 586 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) cited in the application claims 1,2,6,7,9,12 column 1, line 31 column 3, line 56 column 9, line 6	1-14
Y	US 5 468 736 A (HODGEN GARY D) 21 November 1995 (1995-11-21) cited in the application page 1, column 1, line 24,25 column 3, line 56 example 3	1-14
Y	WO 92 18107 A (UNIV SOUTHERN CALIFORNIA) 29 October 1992 (1992-10-29) See estetrol page 23, line 4; claims 1,4,10	1-14
Y	US 5 340 585 A (PIKE MALCOLM C ET AL) 23 August 1994 (1994-08-23) cited in the application column 15, line 1; claims 10,13,14 column 5, line 48-56	1-14
Y	SEEGER H ET AL: "The inhibitory effect of endogenous estrogen metabolites on copper-mediated in vitro oxidation of LDL." INTERNATIONAL JOURNAL OF CLINICAL PHARMACOLOGY AND THERAPEUTICS. GERMANY JUL 1998, vol. 36, no. 7, July 1998 (1998-07), pages 383-385, XP001105329 ISSN: 0946-1965 See "discussion and conclusion" abstract; table 1	1-14
A	HOLINKA C F ET AL: "IN-VIVO EFFECTS OF ESTETROL ON THE IMMATURE RAT UTERUS" BIOLOGY OF REPRODUCTION, vol. 20, no. 2, 1979, pages 242-246, XP001022978 EN ISSN: 0006-3363 * See page 243, materials and methods, compositions comprising estetrol *	1-14

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT 02/00333

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOLINKA C F ET AL: "COMPARISON OF EFFECTS OF ESTETROL AND TAMOXIFEN WITH THOSE OF ESTRIOL AND ESTRADIOL ON THE IMMATURE RAT UTERUS" BIOLOGY OF REPRODUCTION, vol. 22, no. 4, 1980, pages 913-926, XP001037603 ISSN: 0006-3363 * See page 914, materials and methods, compositions comprising estetrol * ---	1-14
A	DE 23 36 434 A (SCHERING AG) 17 April 1975 (1975-04-17) page 4, paragraph 3; claims * See compounds of examples : 6,9-11,16,19-21 * ---	1-14
A	DE 23 36 433 A (SCHERING AG) 3 April 1975 (1975-04-03) page 4, paragraph 3 claims; examples -----	1-14

INTERNATIONAL SEARCH REPORT

International Application No
PCT 02/00333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5827843 A	27-10-1998	NL 9301562 A	03-04-1995
		AU 708881 B2	12-08-1999
		AU 7695294 A	27-03-1995
		EP 0717626 A1	26-06-1996
		FI 961098 A	03-04-1996
		JP 9502194 T	04-03-1997
		AU 1848899 A	29-04-1999
		CA 2171460 A1	16-03-1995
		CN 1133011 A ,B	09-10-1996
		WO 9507081 A1	16-03-1995
		HU 74452 A2	30-12-1996
EP 0136011 A	03-04-1985	US 4826831 A	02-05-1989
		AT 147987 T	15-02-1997
		AU 582540 B2	06-04-1989
		AU 3140584 A	07-02-1985
		CA 1240927 A1	23-08-1988
		DE 3486442 D1	06-03-1997
		DE 3486442 T2	05-06-1997
		DK 377084 A ,B	06-02-1985
		EP 0136011 A2	03-04-1985
		IL 72556 A	15-05-1989
		JP 6035388 B	11-05-1994
		JP 60100520 A	04-06-1985
		LU 90107 A9	17-12-1997
		LU 90343 A9	29-03-1999
		LU 90696 A9	13-02-2001
		LU 90697 A9	13-02-2001
		MX 9203720 A1	31-07-1992
		NZ 209066 A	08-01-1988
		PH 24052 A	05-03-1990
		US RE36247 E	06-07-1999
		ZA 8406077 D	27-03-1985
US 5340586 A	23-08-1994	US 5211952 A	18-05-1993
		US 5340584 A	23-08-1994
		EP 0748191 A1	18-12-1996
		NO 954612 A	12-01-1996
		WO 9426208 A1	24-11-1994
		AT 158717 T	15-10-1997
		CA 2084891 A1	13-10-1992
		DE 69222500 D1	06-11-1997
		DE 69222500 T2	05-02-1998
		DK 538443 T3	14-04-1998
		EP 0538443 A1	28-04-1993
		ES 2109995 T3	01-02-1998
		FI 925652 A	11-12-1992
		NO 924755 A	09-02-1993
		US 5340585 A	23-08-1994
		WO 9218107 A1	29-10-1992
US 5468736 A	21-11-1995	AT 202281 T	15-07-2001
		AU 680239 B2	24-07-1997
		AU 6245494 A	14-09-1994
		BG 62383 B1	29-10-1999
		BG 99880 A	29-02-1996
		BR 9406667 A	23-01-1996
		CA 2157003 A1	01-09-1994

XOCID: <WO_03018026A1_I_>

INTERNATIONAL SEARCH REPORT

International Application No
PCT 02/00333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5468736 A		CZ 9502048 A3	13-03-1996
		DE 69427529 D1	26-07-2001
		DE 69427529 T2	22-11-2001
		DK 686037 T3	27-08-2001
		EP 0686037 A1	13-12-1995
		ES 2159552 T3	16-10-2001
		FI 953926 A	22-08-1995
		GR 3036649 T3	31-12-2001
		HU 72080 A2	28-03-1996
		JP 8510993 T	19-11-1996
		KR 248857 B1	01-04-2000
		NO 953243 A	17-08-1995
		NZ 262657 A	26-05-1997
		PL 310660 A1	27-12-1995
		PT 686037 T	28-09-2001
		RO 115115 B1	30-11-1999
		RU 2139056 C1	10-10-1999
		SK 104995 A3	09-04-1997
		WO 9418983 A1	01-09-1994
WO 9218107 A	29-10-1992	US 5211952 A	18-05-1993
		AT 158717 T	15-10-1997
		CA 2084891 A1	13-10-1992
		DE 69222500 D1	06-11-1997
		DE 69222500 T2	05-02-1998
		DK 538443 T3	14-04-1998
		EP 0538443 A1	28-04-1993
		ES 2109995 T3	01-02-1998
		FI 925652 A	11-12-1992
		NO 924755 A	09-02-1993
		US 5340585 A	23-08-1994
		US 5340586 A	23-08-1994
		WO 9218107 A1	29-10-1992
		US 5340584 A	23-08-1994
US 5340585 A	23-08-1994	US 5211952 A	18-05-1993
		EP 0748190 A1	18-12-1996
		NO 954611 A	16-01-1996
		WO 9426207 A1	24-11-1994
		AT 158717 T	15-10-1997
		CA 2084891 A1	13-10-1992
		DE 69222500 D1	06-11-1997
		DE 69222500 T2	05-02-1998
		DK 538443 T3	14-04-1998
		EP 0538443 A1	28-04-1993
		ES 2109995 T3	01-02-1998
		FI 925652 A	11-12-1992
		NO 924755 A	09-02-1993
		US 5340586 A	23-08-1994
		WO 9218107 A1	29-10-1992
		US 5340584 A	23-08-1994
DE 2336434 A	17-04-1975	DE 2426778 A1	18-12-1975
		DE 2336434 A1	17-04-1975
		AU 7112474 A	15-01-1976
		BE 817599 A1	13-01-1975
		CA 1030950 A1	09-05-1978
		CS 188924 B2	30-03-1979

INTERNATIONAL SEARCH REPORT

International Application No
PCT 02/00333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 2336434 A		DD 114257 A5	20-07-1975
		DD 115658 A5	12-10-1975
		DK 368174 A ,B,	17-03-1975
		ES 428228 A1	01-09-1976
		FI 214074 A ,B,	14-01-1975
		FR 2236504 A1	07-02-1975
		GB 1482285 A	10-08-1977
		IE 39873 B1	17-01-1979
		IL 45260 A	10-03-1978
		JP 50032161 A	28-03-1975
		NL 7409489 A	15-01-1975
		NO 742547 A	10-02-1975
		PL 91361 B1	28-02-1977
		SE 7409152 A	14-01-1975
		US 3951958 A	20-04-1976
		HU 172200 B	28-06-1978
DE 2336433 A	03-04-1975	DE 2336433 A1	03-04-1975
		AU 7112474 A	15-01-1976
		BE 817599 A1	13-01-1975
		CA 1030950 A1	09-05-1978
		DK 368174 A ,B,	17-03-1975
		ES 428228 A1	01-09-1976
		FI 214074 A ,B,	14-01-1975
		FR 2236504 A1	07-02-1975
		GB 1482285 A	10-08-1977
		IE 39873 B1	17-01-1979
		IL 45260 A	10-03-1978
		JP 50032161 A	28-03-1975
		NL 7409489 A	15-01-1975
		NO 742547 A	10-02-1975
		PL 91361 B1	28-02-1977
		PL 92573 B1	30-04-1977
		SE 7409152 A	14-01-1975
		US 3951958 A	20-04-1976
		ZA 7404478 A	30-07-1975

XOCID: <WO__03018026A1_I_>

INTERNATIONAL SEARCH REPORT

Int nal application No.
:T/NL 02/00333

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-14

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

In view of the non-limiting definition "precursors capable of liberating the substance of formula...." in claim 1, present claimsl 1-14 relate to an extremely large number of possible compounds/products. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds explicitly defined by the formula in claim 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-14

   Use of an estrogenic compound falling in the Markush definition given in claim 1 for the manufacture of a pharmaceutical composition for parenteral administration, to be used in association with a progestogenic agent in the treatment of symptoms related to hypoestrogenism.

2. Claims: 15-17

   A drug delivery system for parenteral / rectal administration comprising an estrogenic compound as defined in claim 1, and an androgenic compound.